Maize Nutrient Accumulation and Partitioning in Response to Plant Density and Nitrogen Rate: I. Macronutrients

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ABSTRACT

Understanding nutrient balances in changing cropping systems is critical to appropriately adjust agronomic recommendations and inform breeding efforts to increase nutrient efficiencies. Research to determine the season-long P, K, and S uptake and partitioning dynamics in maize (*Zea mays* L.) as affected by low, medium, and high plant density (PD) and N rate factors and their interactions was conducted over four site-years in Indiana. Plant nutrient contents at maturity responded predominantly to N rate. Relative nutrient contents at silking compared with those at maturity were 47% for P, 100% for K, and 58% for S. Concentrations of P, K, and S varied less in leaf vs. stem (vegetative stage) and in ear vs. shoot (reproductive stage). Equivalent stoichiometric ratios were documented for N and S partitioning in leaf, stem, and ear components. The PD and N rate treatments did not modify P, K, and S nutrient partitioning to plant components during vegetative or reproductive periods (except for an N rate effect on leaf vs. stem P partitioning). Near silking, relative nutrient partitioning to the ear followed the order P > S > K. This mimicked the nutrient harvest indices observed at maturity, suggesting genetic modulation. Ratios of N to P, K, and S in whole-plant tissues were influenced by N content changes in response to N rate but not by PD. As the season progressed, PD and N rates changed the absolute P, K, and S quantities (primarily reflecting biomass responses) but had little influence on nutrient ratios.

ALTHOUGH THE NUTRIENT focus in high-yielding maize production systems is often on N, equally important are advances in our knowledge concerning the uptake quantities and balances of other essential macronutrients, such as P, K, and S. Macronutrient uptake and partitioning (among plant components) of N, P, K, and, to a more limited extent, S have been documented for maize in the older literature (Jones and Huston, 1914; Latshaw and Miller, 1924; Sayre, 1948, 1955; Jordan et al., 1950; Chandler, 1960; Hanway, 1962a, 1962b; Karlen et al., 1987, 1988) and recently by Abendroth et al. (2011). Nevertheless, information for modern hybrids is scarce, and the effect of management practices on the nutrient partitioning process is relatively unknown.

During the last century, maize grain yield (GY) has increased as a result of improvements in both agronomic practices and conventional breeding. From a physiological perspective, the improvements can be attributed to several factors: e.g., greater stress tolerance, especially crowding intensity (Fasoula and Fasoula, 1997; Tokatlidis and Koutroubas, 2004), maintenance of leaf longevity or the stay-green trait (Thomas and Howarth, 2000), effective root systems (Hammer et al., 2009), higher source activity to fulfill ear demand (Tollenaar and Wu, 1999),

and maintenance of individual-plant N uptake with extended reproductive-stage accumulation (Ciampitti and Vyn, 2012, 2013). A contemporary review of long-term studies (Stewart et al., 2005) documented that 57% of the maize GY increase from 1960 to 2000 could be attributed to fertilizer nutrient inputs (N, P, and K fertilizers and lime).

Balanced nutrition must be achieved to optimize maize productivity. More understanding of nutrient balances may be gained by exploring a physiological approach (e.g., uptake and partitioning in different crop growth stages). A tight association between maize productivity and both whole-plant and grain N, P, and K uptake was documented by Setiyono et al. (2010). As GY approached maximum levels, the associations with nutrient uptake tended to plateau. Nevertheless, there are no known prior reports regarding the joint influence of PD and N rates on nutrient uptake and partitioning of other macronutrients in maize plant components with time. Understanding the latter will benefit future GY advancement from both practical and breeding perspectives.

Nutrient content increases as whole-plant biomass (BM) and GY increases (Setiyono et al., 2010), and both BM and GY are very responsive to PD and N rate (Ciampitti and Vyn, 2011). Maize hybrids can vary considerably in their GY response to incremental increases in PD (Tokatlidis et al., 2011). Nonetheless, in a large-scale analysis (subset n = 1972; Setiyono et al., 2010), PD increments were largely unrelated to GY increases (GY 4–19 Mg ha^{-1} for PD range 6–9 plants m^{-2}). A similar wide variation in GY response to fertilizer N rate was also evident, partially explained by differences in soil N supply. Nevertheless, increases in GY were positively associated

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Abbreviations: BM, biomass; GY, grain yield; KHI, potassium harvest index; KIE, potassium internal efficiency; NIE, nitrogen internal efficiency, PD, plant density; PHI, phosphorus harvest index; PIE, phosphorus internal efficiency, RMA, reduced major axis; SHI, sulfur harvest index; SIE, sulfur internal efficiency.

with gains in whole-plant N, P, and K uptake (R^2 = 0.70, 0.38, and 0.46, respectively) (Setiyono et al., 2010). This illustrates the intrinsic complexity of studies on maize PD and N rate interactions and the need for a more thorough examination of the impact of these factors on plant nutrient uptake dynamics.

The primary objective of this study was to understand the P, K, and S content and partitioning dynamics in maize as affected by PD and N rate during the entire season. A second goal was to evaluate macronutrient balances at both the whole-plant and plant-component levels.

MATERIALS AND METHODS

Field experiments were conducted during the 2010 and 2011 seasons at two sites in Indiana: the Purdue University Agronomy Center for Research and Education (40°28′7″ N, 87°0′25″ W; Chalmers silty clay soil, a fine-silty, mixed, superactive, mesic Typic Endoaquoll) and the Pinney–Purdue Agricultural Center (41°26′41″ N, 86°56′41″ W; Sebewa loam soil, a fine-loamy over sandy or sandy-skeletal, mixed, superactive, mesic Typic Argiaquoll). The studies were arranged as a split-split-plot design with six blocks. Three treatment factors were structured by hybrid (main plot: Hybrid 1, Mycogen 2T789; Hybrid 2, Mycogen 2M750—similar comparative relative maturity at 114 d; Dow AgroSciences), PD (subplot: low, medium, and high = 54,000, 79,000, and 104,000 plants ha^{-1}, respectively), and N rate (sub-subplot: low, medium, and high = 0, 112, and 224 kg N ha^{-1} sidedressed, respectively). At planting, all treatments received 25 kg N ha^{-1} (10–34–0 or 19–17–0 as N–P_2O_5–K_2O) as starter fertilizer. The N fertilizer source was urea–NH_4NO_3 (UAN, 28–0–0). For both N sidedress rates, UAN was applied at ∼V6 stage in all site-years. Plots were six rows, 4.5 m wide (76-cm row spacing) and 32 m in length. In all site-years, maize was preceded by soybean [*Glycine max* (L.) Merr.]. Further information can be found in Ciampitti (2012).

Plant Nutrient Content Measurements

Plant BM and nutrient content measurements were determined from the destructive sampling areas as described by Ciampitti (2012). Briefly, BM was determined from six (2010) or 10 to 12 (2011) consecutive plants. Each individual plant was cut at the stem base and separated into different fractions: (i) leaves and stem (vegetative); or (ii) ear and shoot (reproductive). Each fraction was separately chopped and dried to constant weight at 60°C. The P, K, and S concentrations were determined through inductively coupled Ar plasma spectrometry (AOAC International, 2000). Total P, K, and S contents in each fraction were calculated by multiplying each nutrient percentage by the plant BM (dry basis). The nutrient harvest indices (PHI, KHI, and SHI for P, K, and S, respectively) were determined as the ratio between grain and whole-plant P, K, and S contents at maturity (R6 stage).

Plant Nutrient Uptake and Ear Nutrient Allocation Rates

Plant dry mass accumulation rate (PGR) and N, P, K, and S uptake rates around the critical period (CP) bracketing silking (V15–R3 stages, ∼29 d) were calculated as the difference between plant nutrient contents at R3 minus V15 divided by the thermal time between growth intervals using a base temperature of 8°C (Ritchie and NeSmith, 1991). Ear dry mass accumulation

rate (EGR) and N, P, K, and S allocation rates were calculated by dividing the accumulation achieved at R3 by the cumulative thermal time between the R3 and V15 stages (ear biomass = 0 around V15 stage; Otegui and Bonhomme, 1998). At the plant scale, the association between plant and ear dry mass or nutrient allocation was fitted using a linear hyperbolic function (Vega et al., 2001):

$$Y = \frac{\alpha(X - X_0)}{1 + \beta(X - X_0)} \quad [1]$$

where α is the initial slope (maximum dry mass or nutrient partitioning between plant and ear), X_0 is the minimum requirement of plant dry mass or nutrient content to initiate ear dry mass or nutrient allocation, and β is the degree of curvilinearity at greater BM or nutrient uptake levels.

The reproductive partitioning indices for BM and all nutrients were calculated as the ratio between the ear and the whole plant. The ear nutrient gain was calculated as the difference between the ear nutrient content at R6 minus that at R1. Shoot nutrient remobilization was estimated similarly by subtracting the nutrient content in the stem and leaf fractions at R1 from those at R6 (minor nutrient remobilization was quantified before silk emergence; Table 1).

Nutrient Internal Efficiencies

The P, K, and S internal efficiencies (PIE, KIE, and SIE, respectively) were calculated as detailed by Ciampitti and Vyn (2012) for the N internal efficiency (NIE) parameter. As a brief description, nutrient internal efficiency was determined as the grain/whole-plant nutrient content ratio, and each internal efficiency result was divided further into grain nutrient concentration and nutrient harvest index (HI) (Sadras, 2006):

$$\text{Nutrient IE} = \frac{\text{GY}}{\text{nutrient content}}$$
$$= \frac{\text{GY}}{\text{grain nutrient content}} \times \text{nutrient HI} \quad [2]$$

Statistical Analysis

An analysis of variance (ANOVA) was performed using SAS PROC MIXED (SAS Institute, 2004). The analysis was based on the main factors PD and N rate (due to the minor hybrid effect; Ciampitti, 2012). All factors (hybrid, PD, and N rate) including year and site were considered fixed factors (with block as a random factor). For model evaluation, differences between linear functions were tested (F test, Mead et al., 1993), and selected models were compared with a global fit (GraphPad Prism 5; Motulsky and Christopoulos, 2003). For nutrient harvest indices, a correlation analysis was performed using SAS PROC CORR (SAS Institute, 2004).

An allometric evaluation was performed to quantify the association between parameters. Reduced major axis (RMA), derived through the SMATR package (SMATR version 3; Warton et al., 2012) from the R program, was utilized to evaluate the allometric slopes and intercepts. The parameters were $\log_{10}$ transformed ($Y = \alpha X^\beta \rightarrow \log Y = \log\alpha + \beta \log X$) before the analysis (Niklas, 2006), and the normality and residuals distribution were also tested.

Table 1. Leaf and stem P, K, and S contents for different vegetative growth stages (V5, V10, V15, and R1 stages) for maize grown at three plant densities and three N rates (0, 112, and 224 kg N ha^{-1}) across two hybrids, sites, and growing seasons. Only statistically significant levels ($P < 0.05$) for single or interacting main factors (plant density [PD] and N rate [Nr]) resulting from the ANOVA analysis are presented. The standard error (SE) relates only to comparisons between significant terms. At all vegetative stages, plants were fractionated between stem and leaf biomass (i.e., leaf nutrient content and stem nutrient content).

Plant density†	Leaf/stem nutrient contents									
	V5 stage	V10 stage			V15 stage			R1 stage		
	Soil N	0N	112N	224N	0N	112N	224N	0N	112N	224N
	g m^{-2}									
	Phosphorus									
PD1	0.08/0.04	0.51/0.40	0.57/0.38	0.59/0.37	0.53/0.84	0.66/0.94	0.71/0.88	0.54/0.88	0.71/0.91	0.80/0.93
PD2	0.11/0.05	0.57/0.45	0.65/0.44	0.72/0.44	0.61/0.89	0.77/1.00	0.87/0.99	0.57/0.97	0.79/0.99	0.99/1.00
PD3	0.12/0.05	0.61/0.47	0.72/0.43	0.74/0.45	0.64/0.89	0.85/0.98	0.92/0.92	0.63/1.03	0.91/1.05	1.05/0.97
ANOVA	PD	PD, Nr/PD			PD, Nr/Nr			PD × Nr/PD		
SE	0.006/0.003	0.012/0.011			0.016, 0.014/0.029			0.025/0.024		
	Potassium									
PD1	0.64/0.40	4.20/6.59	4.39/7.05	4.49/7.12	4.70/11.70	5.23/13.24	5.5/13.46	4.65/10.49	5.49/12.50	5.76/13.25
PD2	0.90/0.57	5.01/7.94	5.40/8.51	5.53/8.52	5.65/12.75	6.49/16.08	6.66/14.88	5.39/11.95	6.67/14.87	7.20/14.87
PD3	1.04/0.59	5.60/8.83	6.15/8.93	6.16/9.45	6.18/13.40	7.64/17.57	7.57/16.59	6.20/13.41	7.82/16.89	8.31/16.30
ANOVA	PD	PD, Nr/PD, Nr			PD × Nr/PD × Nr			PD × Nr/PD, Nr		
SE	0.04/0.04	0.08/0.18			0.13/0.48			0.18/0.30		
	Sulfur									
PD1	0.05/0.01	0.31/0.12	0.35/0.14	0.37/0.14	0.34/0.24	0.44/0.31	0.46/0.32	0.39/0.22	0.51/0.28	0.56/0.33
PD2	0.07/0.02	0.34/0.13	0.39/0.15	0.45/0.17	0.39/0.24	0.51/0.33	0.55/0.35	0.41/0.24	0.55/0.30	0.66/0.36
PD3	0.09/0.02	0.37/0.14	0.46/0.16	0.47/0.18	0.40/0.24	0.56/0.34	0.59/0.35	0.45/0.27	0.65/0.35	0.70/0.37
ANOVA	PD	PD, Nr/PD, Nr			PD × Nr/PD, Nr			PD × Nr/PD, Nr		
SE	0.003/0.001	0.008/0.005			0.015/0.009			0.015/0.004		

† PD1, low = 54,000 plants ha^{-1}; PD2, medium = 79,000 plants ha^{-1}; PD3, high = 104,000 plants ha^{-1}.

RESULTS AND DISCUSSION
Nutrient Content Evolution: Phosphorus, Potassium, and Sulfur

Phosphorus and S evolution followed a similar pattern to that previously documented for N and whole-plant BM (Ciampitti, 2012; also presented in Fig. 1J–1O). The proportion of vegetative-stage nutrient content (relative to the total at maturity) varied with the PD factor (45–50% for P and 56–61% for S from low to high PD levels; Fig. 1). The K content showed a distinctive pattern, with maximum K at R3 (Fig. 1D–1F). The soil P and K levels (averaging, across four site-years, ~64 mg P kg^{-1} and 147 mg K kg^{-1}, both determined by Mehlich-3) were well above critical limits. In addition, an average of 23 kg ha^{-1} of P (range 10–37 kg P ha^{-1}) was banded as a starter fertilizer at planting. It was, therefore, unlikely that plant uptake of these nutrients was constrained by soil deficiencies.

For P, the highest P uptake rate occurred around silking (~V15–R3; 4.1 mg m^{-2} °C^{-1} d^{-1}). The cumulative P uptake was significantly influenced by PD and N rate from early vegetative to silk emergence, after which the PD effect (but not the N effect) disappeared (Table 1; Fig. 1A–1C). The P and S uptake responded to N rate similarly to the response documented for BM and N content (Fig. 1J–1O). Across treatments, the quantity of P stored was 2.1 g m^{-2} at silking and 4.6 g m^{-2} at maturity (mean GY ~10 Mg ha^{-1}; Ciampitti, 2012). From a compendium of data sets, Setiyono et al. (2010) reported a mean P content of 3.5 g m^{-2} at maturity (GY ~12 Mg ha^{-1}). Similar values ranging from 2.6 to 4.9 g m^{-2} (GY ~ 9–16 Mg ha^{-1}) were reported by Wortmann et al. (2009), Djaman (2011), and Peng et al. (2012).

The maximum K uptake rate was observed between V5 and V15 phases, with a mean K uptake rate of 41.5 mg m^{-2} °C^{-1} d^{-1}. Potassium uptake was significantly affected by PD and N rate

during the entire season (Fig. 1D–1F). Overall, the K content averaged ~13 g m^{-2} during early vegetative development (V10), reached a maximum at R3 (~23 g m^{-2}), and declined to maturity (to reach ~18 g m^{-2}). Similar late-season K declines were documented by Jones and Huston (1914), Sayre (1948), Hanway (1962a), Karlen et al. (1988), Djaman (2011), and Ning et al. (2012). In addition, Mallarino et al. (2011) quantified K and P losses from maize vegetative structures between maturity and harvest. Setiyono et al. (2010) reported a mean K of 26.9 g m^{-2} at maturity (range 0.43–59.8 g m^{-2}). Even higher K and P contents (>30 g K m^{-2} and >6 g P m^{-2}) were reported with superior GYs (~14 Mg ha^{-1}; Singer et al., 2007).

Similarly to P uptake, the highest S uptake rate was recorded around silking (1.7 mg m^{-2} °C^{-1} d^{-1}; ~V15–R3), with the greatest uptake in the interval from silking to R3. This also mimics the BM and N content patterns (Fig. 1J–1O). Sulfur uptake was influenced by PD and N rate only during the vegetative period; during the reproductive stage, the uptake rate was modified only by the N rate (Fig. 1G–1I). Across treatments, the S stored was 1.0 g m^{-2} at silk emergence and 1.7 g m^{-2} at maturity. Rabuffetti and Kamprath (1977) and Pagani et al. (2009) reported similar total S contents (range 1.0–2.5 g m^{-2}) with GYs ranging from 6 to 14 Mg ha^{-1}.

At the whole-plant level, the dilution functions for each nutrient (N [from Ciampitti et al., 2012], P, K, and S) showed that S followed the N dilution model and was largely influenced by the N rate, while P and K (large variation) were relatively unmodified by PD or N rate (Fig. 2). Both Plénet and Lemaire (1999) and Ziadi et al. (2007) reported, in general, that neither N nor P deficiencies severely reduced maize BM and P concentration compared with optimum N or P. Ranges in plant P and/or K dilution in our research were similar to those

reported by Hanway (1962b), Heckman and Kamprath (1992), Mallarino (1996), and Mallarino and Higashi (2009). Under severe N deficiency (>50% reduction in BM), P and K dilutions were fitted by two models (0 N vs. 67–134 kg N ha^{-1}) (Jordan et al., 1950). Further details related to nutrient dilution models can be found in Terman and Noggle (1973).
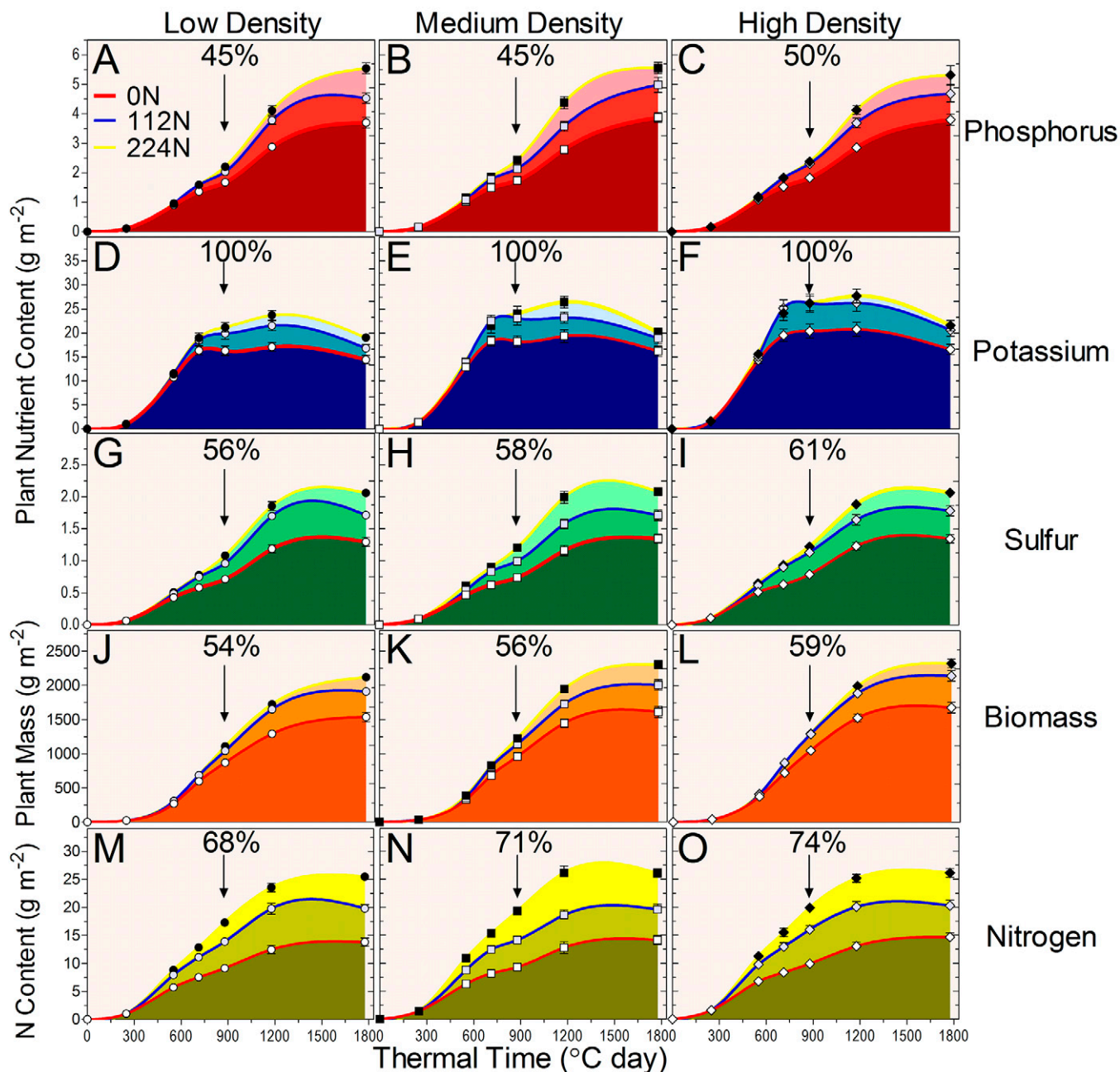
Despite occasional discrepancies, similar nutrient content evolution trends (as the maize crop aged) were reported for P, K, and S by other researchers (Jones and Huston, 1914; Sayre, 1948; Hanway, 1962a; Karlen et al., 1988; Pagani et al., 2009; Djaman, 2011; Peng et al., 2012). When averaged across a wide range of PD and N treatments for four site-years, our research shows that maize accumulated 47% of the total BM, 38% of the total N, 42%

of the P, 47% of the S, and 10% of the K content (Fig. 1) during the period bracketing silking (or about one-fourth of the entire growing season duration expressed on a thermal time basis).

Plant Components: Phosphorus, Potassium, and Sulfur

A separate analysis was performed for the plant fractions evaluated during the vegetative (leaf and stem) and reproductive (shoot and ear) periods (Tables 1 and 2). Leaf and stem P, K, and S contents increased as the crop developed (BM increased).

For P, the leaf/stem content ratio was greatest during early vegetative growth (V5–V10; 1.8 leaf/stem P ratio) but trended lower during late vegetative stages (V15–R1; 0.8 leaf/stem P ratio).

Fig. 1. Nutrient contents for (A,B,C) P, (D,E,F) K, and (G,H,I) S vs. thermal time (°C d) after emergence for maize plants at three plant densities (low, 54,000 [circles]; medium, 79,000 [squares]; and high, 104,000 [diamonds] plants ha^{-1}) and three N rates (0, 112, and 224 kg N ha^{-1} sidedressed) across two hybrids, sites, and growing seasons. The arrows on each panel indicate the average silking date across the treatment combinations. Percentages are proportions (averaged across three N rates) of vegetative-stage nutrient contents relative to total accumulations at maturity. Curves were fitted using Gompertz equations; (J,K,L) biomass and (M,N,O) N content evolution were reported by Ciampitti (2012).

In addition, leaf and stem P contents consistently increased as the PD increased (Table 1). During the vegetative phase, the leaf P content was more responsive than stem P content to the rate of fertilizer N applied (Table 1). For K, the leaf K content relative to the stem was greatest at V5 (overall 1.7 leaf/stem K ratio), declined as the crop aged, and reached an overall leaf/stem ratio of 0.5 at silk emergence (Table 1). At the late-vegetative stage, leaf K content responded more ($\sim$24%) to N applied (0 vs. 224 kg N ha^{-1}) than did the stem ($\sim$19%), although the overall stem content was higher. For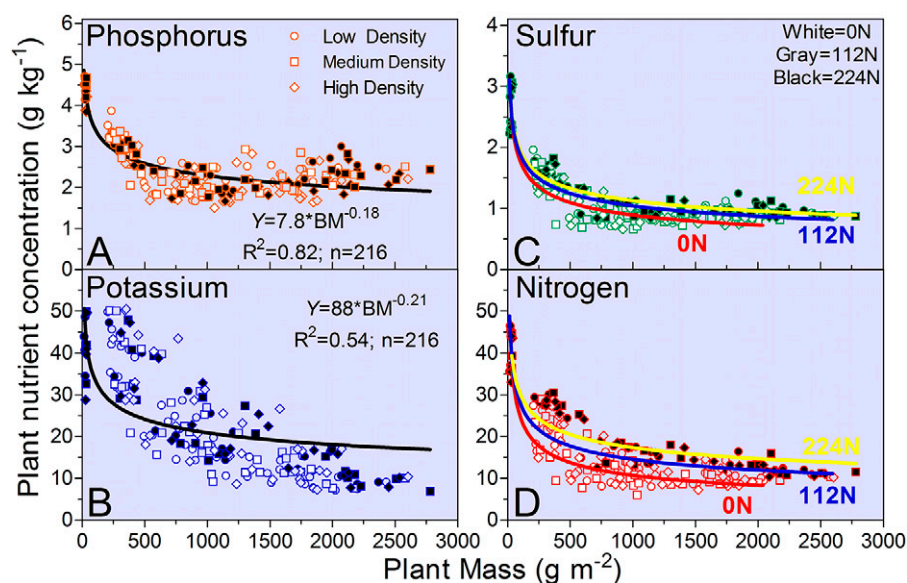 S, a consistently greater leaf S vs. stem S content was observed during the entire vegetative period, although the ratio itself declined toward silk emergence (3.5 to 1.7 leaf/stem S ratio). At the late-vegetative stage, S content in the leaf responded to N fertilization (0 vs. 224 kg N ha^{-1}) more ($\sim$35%) than the stem ($\sim$30%) (Table 1).

During the reproductive period, higher N rates were reflected in concomitant increases in P, K, and S contents in the ear, while the PD effect showed an opposite trend, declining as the crowding stress was intensified (Table 2). This PD effect was highly dependent on ear mass differences (high PD, small ear
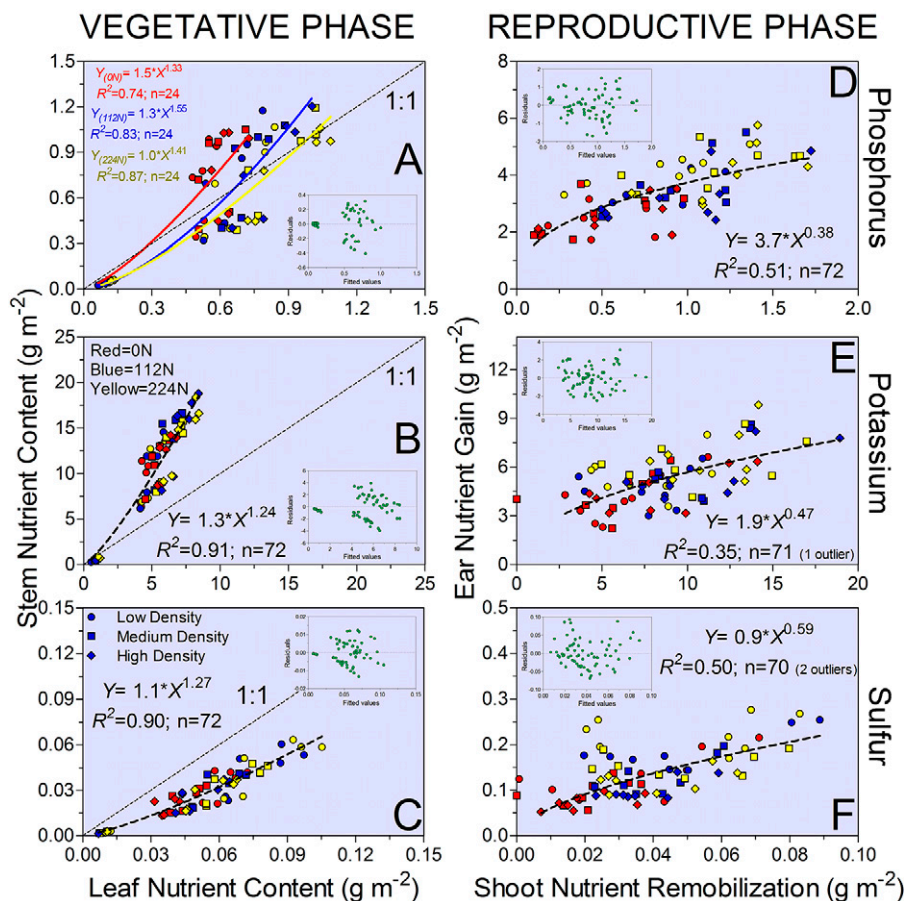
Table 2. Shoot and ear P, K, and S contents for reproductive growth stages (R1, R3, and R6) for maize at three plant densities and three N rates (0, 112, and 224 kg N ha^{-1} sidedressed) across two hybrids, sites, and growing seasons. Only statistically significant levels ($P < 0.05$) for single or interacting main factors (plant density [PD] and N rate [Nr]) resulting from the ANOVA analysis are presented. The standard error (SE) relates only to comparisons between significant terms. At all reproductive stages, plants were fractionated between shoot (leaf plus stem) and ear biomass (i.e. shoot N content and ear N content). Fractionation of the ear varied at different growth stages (at the R1 stage, no fractionation; at R3, fractionation into husk and cob plus grain; at R6, fractionation into grain, cob, and husk).

Plant density†	R1 stage (ear)			R3 stage (shoot/husk/cob + grain)			R6 stage (shoot/husk/cob/grain)		
	0N	112N	224N	0N	112N	224N	0N	112N	224N
						g m^{-2}			
						P uptake			
PD1	0.26	0.42	0.47	1.56/0.12/1.20	1.79/0.15/1.84	1.97/0.17/1.97	0.94/0.04/0.06/2.66	0.83/0.04/0.06/3.59	0.87/0.05/0.06/4.57
PD2	0.20	0.36	0.45	1.57/0.11/1.11	1.70/0.14/1.74	2.09/0.15/2.13	1.03/0.04/0.07/2.73	0.85/0.05/0.07/4.02	0.81/0.05/0.06/4.64
PD3	0.17	0.35	0.35	1.71/0.11/1.04	1.91/0.14/1.64	2.01/0.15/1.96	1.09/0.04/0.07/2.60	0.94/0.05/0.08/3.62	0.85/0.04/0.06/4.36
ANOVA		PD, Nr			Nr/PD, Nr/PD × Nr (P = 0.064)			Nr/PD × Nr/PD × Nr/Nr	
SE		0.01			0.055/0.005/0.064			0.05/0.002/0.003/0.11	
						K uptake			
PD1	1.20	1.91	2.24	13.2/0.65/3.24	16.2/0.95/4.38	17.8/1.11/4.83	9.27/0.56/0.83/3.84	10.3/0.73/0.89/4.97	11.4/0.88/0.81/6.00
PD2	0.97	1.60	2.04	15.6/0.61/3.2	18.0/0.8/4.46	20.1/0.98/5.42	10.9/0.51/1.03/3.86	11.5/0.71/1.2/5.54	11.7/0.84/1.26/6.35
PD3	0.83	1.58	1.67	17.1/0.56/3.17	21.1/0.76/4.45	21.7/0.89/5.23	11.3/0.49/1.11/3.70	13.5/0.61/1.36/5.15	13.5/0.71/1.34/6.06
ANOVA		PD, Nr			PD, Nr/PD, Nr/Nr			PD × Nr/PD × Nr (P = 0.06)/PD × Nr/Nr	
SE		0.06			0.46/0.02/0.09			0.45/0.02/0.03/0.12	
						S uptake			
PD1	0.10	0.17	0.20	0.72/0.04/0.42	1.00/0.06/0.63	1.10/0.07/0.69	0.43/0.04/0.04/0.78	0.51/0.05/0.05/1.11	0.62/0.05/0.05/1.35
PD2	0.08	0.14	0.18	0.71/0.05/0.41	0.94/0.06/0.58	1.18/0.07/0.74	0.48/0.04/0.06/0.77	0.52/0.04/0.05/1.1	0.62/0.04/0.06/1.36
PD3	0.07	0.14	0.15	0.80/0.04/0.39	1.00/0.06/0.58	1.13/0.07/0.69	0.50/0.04/0.06/0.75	0.59/0.04/0.07/1.08	0.66/0.04/0.06/1.31
ANOVA		PD, Nr			Nr/Nr/PD × Nr			PD, Nr/PD × Nr (P = 0.14)/PD × Nr/Nr	
SE		0.05			0.03/0.07/0.02			0.01/0.001/0.002/0.01	

† PD1, low = 54,000 plants ha^{-1}; PD2, medium = 79,000 plants ha^{-1}; PD3, high = 104,000 plants ha^{-1}.

Fig. 2. Relationship between plant nutrient concentration for (A) P, (B) K, (C) S, and (D) N vs. plant biomass (BM) from the V5 growth stage until the end of the season (R6 stage) evaluated at three plant densities (low, 54,000 [circles]; medium, 79,000 [squares]; and high, 104,000 [diamonds] plants ha^{-1}) and three N rates (0, 112, and 224 kg N ha^{-1} sidedressed) across two hybrids, sites, and growing seasons. Each observation represents an average of three replications for each treatment across hybrid levels (average of two hybrids), totaling $n = 216$ ($n = 36$ data points for each phenological stage evaluated: nine plant density × N rate combinations at four site-years). For S, fitted equations were: Y (0N) = $7.0BM^{-0.30}$ (red), Y (112 N) = $6.1BM^{-0.26}$ (blue), Y (224N) = $5.7BM^{-0.24}$ (yellow), all R^2 values >0.88, $n = 72$. For N, fitted equations were: Y (0N) = $132BM^{-0.36}$ (red), Y (112N) = $104BM^{-0.28}$ (blue), Y (224N) = $91BM^{-0.24}$ (yellow), all R^2 values >0.86, $n = 72$. Evolution in N concentration was previously reported by Ciampitti (2012).

In figure panels:
- Phosphorus: $Y = 7.8*BM^{-0.18}$, $R^2 = 0.82$; $n = 216$
- Potassium: $Y = 88*BM^{-0.21}$, $R^2 = 0.54$; $n = 216$

VEGETATIVE PHASE **REPRODUCTIVE PHASE**

Fig. 3. (A,B,C) Stem vs. leaf nutrient contents (P, K, and S) (V5–R1 growth stages) and (D,E,F) ear nutrient gain (grain, cob, and husk fractions) vs. shoot (stem plus leaf components) nutrient remobilization (R1–R6 growth stages) for maize hybrids evaluated at three plant densities (low, 54,000 [circles]; medium, 79,000 [squares]; and high, 104,000 [diamonds] plants ha^{-1}) and three N rates (0, 112, and 224 kg N ha^{-1} sidedressed) across two hybrids, sites, and growing seasons. Each data point represents the plant density and N rate effects across hybrids for each growth stage. Insets show the residuals distribution for each association evaluated (residuals vs. fitted values).

sizes). At the 0 N rate, ear BM was much reduced at maturity (Ciampitti, 2012), with consequently lower ear P, K, and S contents compared with when sidedress N was applied. During the reproductive period, a narrow ear variation was apparent for S and P concentrations [∼1.5 and 2.7, calculated as variation = (maximum – minimum)/minimum; Sadras, 2007], while the shoot showed a larger variation range (∼9 for S and 12 for P). In contrast, the K concentration showed a similar variation in both shoot and ear plant fractions (∼8.6 and ∼7.1). Shoot K content decreased as the reproductive phase progressed (presumably explained by nutrient remobilization, K losses via leaf senescence, and leaching from vegetative structures) and as the ear K content increased. At maturity, P, K, and S contents in the maize grain were positively affected by the N rate. Similar N rate effects on maize grain N, P, and K were previously documented (Hanway, 1962b; Arnold et al., 1977; Feil et al., 1993; Alfoldi et al., 1994).

Two points are noteworthy for all nutrients: (i) shoot nutrient concentrations declined rapidly from the R1 to R3 stages, and (ii) at different ear BM, the ear nutrient concentration displayed a narrow variation range, thus reflecting a conservative feature (similar to the leaf nutrient concentration during the vegetative phase).

To further investigate the nutrient trends, study of the association between leaf and stem fractions was pursued (Fig. 3). In our previous research, a proportional association was documented between the stem and leaf dry mass allocation across all treatments (Ciampitti, 2012). Thus, management practices introduced modifications in plant size without promoting shifts in the plant morphology. Briefly, for the nutrient associations between leaf and stem fractions, three points should be highlighted: (i) P demonstrated a close isometry for nutrient contents in both

components (partitioning influenced by the N rates); (ii) K showed proportionally more nutrient allocation to the stem than the leaf; and (iii) S was allocated more to the leaf than the stem as the nutrient content increased during the vegetative phase (Fig. 3A–3C). These trends were confirmed by the RMA analysis (log–log scale; Supplementary Table 1), demonstrating a lack of significant PD and N rate effects (except for P). In addition, at comparable stem P concentrations, the leaf P concentration followed the N rate order 224 > 112 > 0 kg N ha^{-1} (Fig. 3B).

Associations between ear nutrient gain and shoot nutrient remobilization were evaluated during the reproductive period (Fig. 3D–3F). For P, K, and S, the general trend was for a higher quantity to be remobilized as the ear BM increased regardless of the PD and N rate treatments (unique fitted models). The log–log analysis (Supplementary Table 1) confirmed that the remobilization ratio was unaffected by the management practices used. The variation in the ear nutrient gain accounted for by nutrient remobilization was moderate for P and S ($R^2 \sim$ 0.5) but low for K ($R^2 < 0.4$) (Fig. 3D–3F), indicating that shifts in shoot nutrient content from R1 to R6 were affected by more than just remobilization to the grain (e.g., leaching losses).

Nutrient Partitioning Ratios between Vegetative and Reproductive Structures

The log–log nutrient partitioning study enabled a further understanding and statistical quantification of the management practices effects. Associations between the leaf, stem (four growth stages = V5, V10, V15, and R1), and ear (three growth stages = R1, R3, and R6) nutrient contents and biomasses (slopes represent nutrient concentration for each component) were

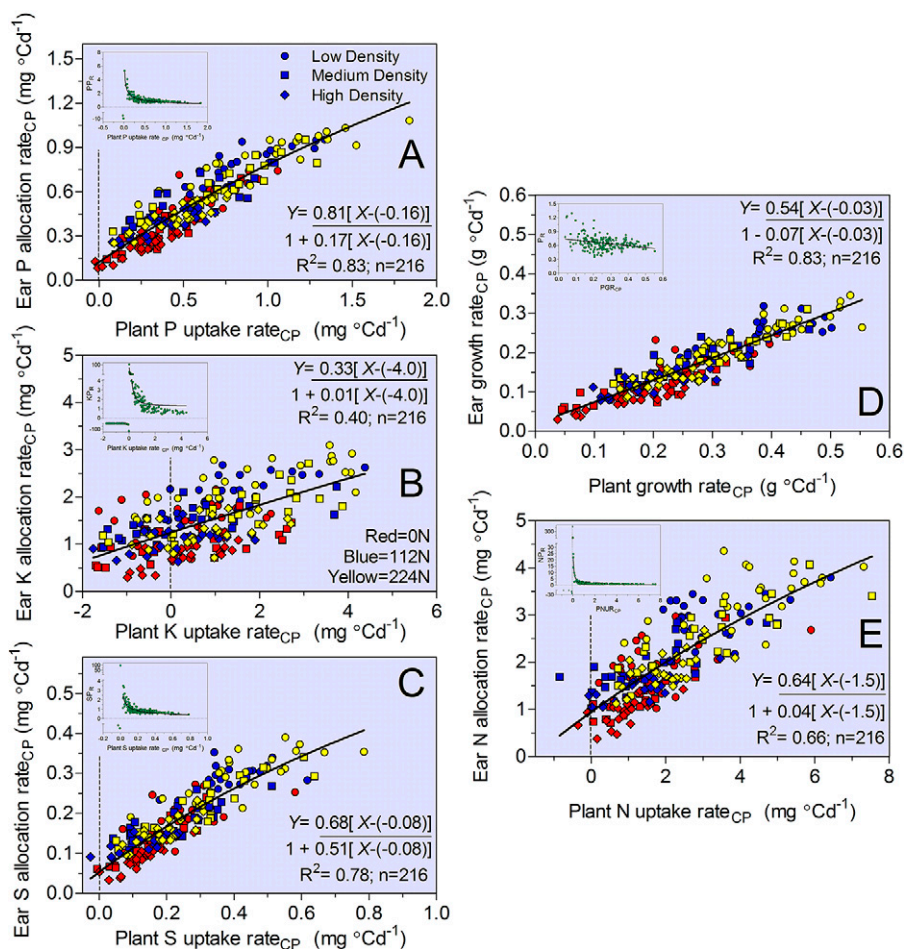| Plant density†	PUR			EAR			HI			Grain concentration			IE		
	0N	112N	224N	0N	112N	224N	0N	112N	224N	0N	112N	224N	0N	112N	224N
	——— mg °C d⁻¹ ———									—— g kg⁻¹ dry mass ——			—— kg kg⁻¹ ——		
Phosphorus															
PD1	0.59	0.86	1.00	0.52	0.78	0.84	0.71	0.79	0.82	3.4	3.5	4.0	215	247	230
PD2	0.35	0.49	0.68	0.33	0.51	0.61	0.70	0.80	0.83	3.7	3.9	3.7	186	224	245
PD3	0.28	0.39	0.48	0.24	0.37	0.44	0.68	0.77	0.81	3.6	3.5	3.6	173	242	250
ANOVA															
PD		*** (0.030)‡			*** (0.014)			* (0.008)			ns (0.1)			ns (6.9)	
Nr		*** (0.025)			*** (0.012)			*** (0.007)			ns (0.09)			*** (7.1)	
PD × Nr		** (0.038)			** (0.019)			ns (0.011)			ns (0.2)			* (10.4)	
Potassium															
PD1	0.31	1.27	1.95	1.53	2.09	2.33	0.27	0.30	0.32	4.9	4.9	5.1	56.0	66.9	67.2
PD2	0.32	0.25	1.41	1.02	1.43	1.71	0.25	0.30	0.33	5.1	5.3	5.1	45.6	59.7	68.7
PD3	0.29	0.26	0.79	0.78	1.09	1.28	0.23	0.25	0.29	5.0	4.9	4.9	38.9	54.8	60.5
ANOVA															
PD		** (0.14)			*** (0.027)			*** (0.005)			ns (0.1)			*** (1.6)	
Nr		*** (0.15)			*** (0.024)			*** (0.005)			ns (0.09)			*** (1.5)	
PD × Nr		** (0.20)			** (0.040)			ns (0.008)			ns (0.2)			** (2.1)	
Sulfur															
PD1	0.24	0.37	0.43	0.18	0.27	0.30	0.59	0.65	0.66	1.0	1.1	1.2	617	639	613
PD2	0.14	0.20	0.30	0.12	0.17	0.22	0.57	0.63	0.65	1.0	1.0	1.1	527	641	647
PD3	0.13	0.16	0.20	0.09	0.13	0.16	0.54	0.60	0.63	1.0	1.0	1.1	458	599	599
ANOVA															
PD		*** (0.011)			*** (0.004)			*** (0.004)			ns (0.01)			** (13.1)	
Nr		*** (0.010)			*** (0.003)			*** (0.003)			ns (0.01)			*** (13.0)	
PD × Nr		** (0.017)			*** (0.006)			ns (0.006)			* (0.02)			** (19.6)	

investigated (Supplementary Fig. 1). In addition to the P, K, and S data sets, the leaf, stem, and ear N contents were also evaluated from the data summarized by Ciampitti (2012). The following points are noteworthy: (i) as the logarithmic component mass increased, the logarithmic nutrient contents also increased; (ii) changes in PD did not result in any significant shifts (unique slopes) in nutrient partitioning; (iii) the N rate affected the nutrient partitioning slopes (0 vs. 112–224 kg N ha⁻¹) for N, P, and S in the leaf and for N and S in the stem but did not affect partitioning of any of the three nutrients in the ear (Supplementary Fig. 1; Table 2); and (iv) the logarithmic nutrient content/logarithmic component mass ratio was equivalent for N and S for the leaf, stem, and ear plant fractions, while P showed a greater within-organ partitioning than N and S in all plant components (Supplementary Fig. 1).

Two specific timings were also evaluated in the log–log analyses: (i) at physiological maturity, evaluating the relationship between grain and shoot nutrient partitioning and its relationship with each component mass and the whole-plant BM; and (ii) during the time frame around silking (±15 d) due to its importance in the nutrient content and BM accumulation trends.

At maturity, the log–log analyses of the grain nutrient content vs. component mass (Supplementary Fig. 1D; Table 3) demonstrated that P, K, and S had equivalent proportional partitioning ratios (P = K = S slopes), while N was higher

(greater slope). In the shoot fraction at maturity (Supplementary Fig. 1E), N and K were equivalent (similar to S); however, the shoot P content did not change as shoot BM increased (conservative shoot P concentration characteristic). Instead, grain was the primary sink for P, demonstrated by the high grain/shoot ratios (Supplementary Fig. 1F). The S grain/shoot ratio was very close to those for N and P, increasing as BM increased at maturity. The final grain/shoot nutrient ratios increased proportionally more for N and P than for S and K as the total crop BM increased (Supplementary Fig. 1F).

The concept of evaluating nutrient partitioning between plant and ear fractions around the silking period is not novel (it was first proposed for dry matter partitioning by Andrade et al. [1999] and Vega et al. [2001]). Nonetheless, there are no known published studies regarding N, P, K, and S nutrient partitioning from plant to ear components. Overall, the most important findings from this analysis (Fig. 4) are: (i) the allometric relationships between ear and plant for dry mass, N, P, K, and S contents were unaltered by the treatments evaluated; (ii) higher values for ear and plant dry mass and nutrient content were primarily associated with a decrease in PD and an increase in the N rate applied; (iii) the reproductive partitioning ratios, ranging in order from high to low, were P > S > N > dry mass > K, but these ratios decreased as dry mass or nutrient content increased (insets in Fig. 4); (iv) interestingly enough, the maximum partitioning indices (α and

Fig. 4. Nutrient allocation from the total plant to the ear component for (A) P, (B) K, and (C) S during the critical period bracketing silking (~29 d, ~464°C d) evaluated at three plant densities (low, 54,000 [circles]; medium, 79,000 [squares]; and high, 104,000 [diamonds] plants ha⁻¹) and three N rates (0 , 112, and 224 kg N ha⁻¹ sidedressed) across two hybrids, sites, and growing seasons. Insets show the association between (A) the P reproductive partitioning index (PP$_R$, ear allocation rate/plant P uptake rate [PPUR] ratio) and the PPUR in the critical period bracketing silking (PPUR$_{CP}$); (B) the K reproductive partitioning index (KP$_R$) and the plant K uptake rate for the critical period (PKUR$_{CP}$); and (C) the S reproductive partitioning index (SP$_R$) and the plant S uptake rate for the critical period (PSUR$_{CP}$). Each observation represents a replication; (D) dry mass growth and (E) N partitioning based on information reported by Ciampitti (2012).

initial slopes, Fig. 4) were coincident with the mean grain and nutrient harvest indices at maturity, suggesting strong genetic control in the early reproductive stages; (v) for all relationships, no minimum requirement was needed (X_0 was negative in all relationships) for the onset of dry mass and nutrient allocation to the ear; and (vi) the degree of curvilinearity (β) was greater for S and P but more linear for dry mass, N, and K (suggesting a potential for further improvement in the maximum values; Fig. 4).

Hybrid variation for the dry mass relationship has been previously reported (Echarte et al., 2004; Luque et al., 2006; D'Andrea et al., 2008), but the hybrids evaluated in this research were not substantially different in either ear or plant components (<10% variation). It can be postulated that more substantive genetic differences in the plant and ear dry mass accumulation rates (e.g., more contrasting genotypes) will be more likely to change the N, P, and S patterns. Thus, genotypes with a greater dry mass requirement to onset the allocation of dry mass to the ear will show similar behavior for N, P, and S. Lastly, it can also be hypothesized that nutrient allocation and uptake processes were driven by the sink strength (ear size and demand). Peng et al. (2012) also confirmed the primary role of sink demand, as distinct from root system size, in plant N, P, and K uptake.
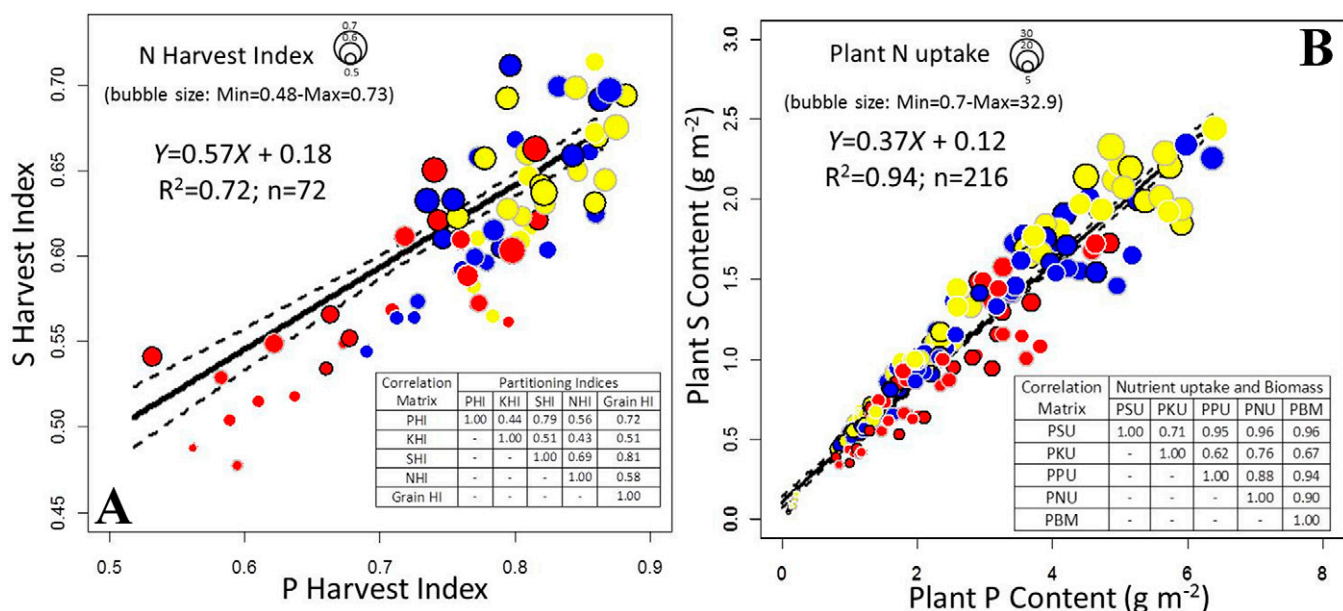
Dissecting the Internal Efficiencies: Grain Nutrient Concentrations and Harvest Indices

The nutrient internal efficiencies for P (PIE), K (KIE), and S (SIE) were calculated (GY to nutrient content), and the grain nutrient concentrations (Pg, Kg, and Sg) and harvest indices were also evaluated (Table 3). Both PD and N rate significantly

influenced all internal efficiencies (Table 3). Mean PIE was 224 kg kg⁻¹, and maximum PIE occurred at the highest PD and N rate levels (~250 kg kg⁻¹). Previously reported mean PIE values ranged from ~260 to 405 kg kg⁻¹ (Wang et al., 2007; Parentoni and Lopes de Souza, 2008; Setiyono et al., 2010; Ning et al., 2012). In our case, PIE was primarily driven by changes in Pg (PIE decreased as Pg increased) and, to a lesser extent, by the PHI (PIE increased as the PHI rose; Table 3). Similar associations were documented for the NIE (Ciampitti and Vyn, 2011, 2012). The Pg (mean = 3.6 g kg⁻¹) was very stable across treatment levels (without significant treatment effects; Table 3). Across 23 site-years, similar maize Pg (mean = 4.0 g kg⁻¹) was reported by Heckman et al. (2003) at a mean GY of 10.3 Mg ha⁻¹. Across 25 site-years in Iowa, Mallarino (1996) reported a mean maize Pg of 2.8 g kg⁻¹ across four P rates (GY = 9.1 Mg ha⁻¹). The shoot P concentration (mean = 1.2 g kg⁻¹) followed a plateau trend as the shoot mass increased (slope, Fig. 4E). Similar mean shoot P concentrations (1.2 g kg⁻¹) and grain P concentrations (3.0 g kg⁻¹) were reported by Mallarino et al. (2011) across 11 maize site-years (GY = 10.7 Mg ha⁻¹).

The mean KIE was 57.6 kg kg⁻¹, and its maximum level (69 kg kg⁻¹) occurred with the medium PD and 224 kg N ha⁻¹ treatment combination, which also coincided with the maximum KHI (Table 3). A similar mean KIE (51 kg kg⁻¹) was reported by Setiyono et al. (2010). The KIE responded positively to higher N rates and generally increased as the PD decreased. In contrast with P, no trends were observed between KIE and Kg but there was a strong association between KIE and shoot K concentration. In addition, Kg was not significantly influenced

Fig. 5. (A) Sulfur vs. P harvest indices (SHI and PHI, respectively) as related to the N harvest index (NHI) determined at physiological maturity, with the area of the circles corresponding to NHI values ranging from 0.48 to 0.73, and (B) whole-plant S and P contents associated with the N content during the entire maize growing season, with the area of the circles corresponding to plant N uptake ranging from 0.7 to 32.9 g m^{-2}, evaluated at three plant densities (low, 54,000 [black outlines]; medium, 79,000 [gray outlines]; and high, 104,000 plants ha^{-1} [white outlines]) and N rates (0 [red], 112 [blue], and 224 kg N ha^{-1} [yellow] sidedressed) across two hybrids, sites, and growing seasons. Each data point represents the average of three replications for each site-year, two locations, and two seasons (n = 72); for (B), each observation refers to the plant density × N rate interaction across hybrids for the four site-years. The data for grain harvest index (HI) and NHI were obtained from Ciampitti (2012). Dotted lines indicate the 95% confidence interval for the fitted linear model.

by PD or N rate (Table 3). A strong positive linear trend was found between KIE and KHI (data not shown). Grain K (mean = 5 g kg^{-1}) varied much less (3–8 g kg^{-1}) than the shoot K (mean = 15 g kg^{-1}, range 7–25 g kg^{-1}; Table 3). Heckman et al. (2003) also reported a similar mean and variation range for Kg (mean = 4.8, range 3.1–6.2 g kg^{-1}), while Mallarino and Higashi (2009) documented a lower Kg mean and narrower variation (mean = 3.5, range 2.8–4.4 g kg^{-1}, GY 9.3 Mg ha^{-1}) across K rates and site-years.

The maximum SIE was achieved at medium PD and the highest N rate (647 kg kg^{-1}). Both PD and N rate factors influenced SIE. Similar SIE values can be calculated from Rabuffetti and Kamprath (1977), with a mean of 541 kg kg^{-1} (range 403–825 kg kg^{-1}) and a tendency to greater SIE with lower Sg (when N and S fertilizers were not applied). In addition, Wortmann et al. (2009) documented an average SIE of 636 kg kg^{-1} (high GY level, ∼14 Mg ha^{-1}). The SHI followed a positive (but weak) association with SIE, while the Sg was negatively associated with SIE. Our mean Sg was 1.1 g kg^{-1} (Table 3), coincident with the average documented by Rabuffetti and Kamprath (1977) and Heckman et al. (2003). Although Sg variation was low, it was significantly affected by a PD × N rate interaction (Table 3), as Sg was maximized at 224 kg N ha^{-1} and the lowest PD level.

Nutrient harvest indices, PHI (mean = 0.78), KHI (mean = 0.28), and SHI (mean = 0.61), increased as the PD was reduced and the N rate increased (Table 3). Setiyono et al. (2010) reported a mean PHI of 0.84 and KHI of 0.17. Similar values were documented by Hanway (1962a) and Mallarino (1996). Recently, Ning et al. (2012) reported an average PHI of 0.67 and KHI of 0.25. Rabuffetti and Kamprath (1977) reported an average SHI of 0.60 (GY ∼ 6–7 Mg ha^{-1}). For PHI, Sinclair and Vadez

(2002) suggested that the partitioning of P to the grain is unlikely to increase beyond 0.8. The association between PHI and SHI (with NHI expressed via the bubble sizes) increased with the grain harvest index (inset correlation matrix, Fig. 5A). Thus, as documented for NHI in the comprehensive review by Ciampitti and Vyn (2012), the high correlations among all dry mass and nutrient partitioning indices suggest additional challenges in breeding efforts directed at a specific nutrient trait stability (expressed as high partitioning) under low GY levels.

Following sidedress N (112–224 kg N ha^{-1}), PHI mostly ranged from 0.70 to 0.85, SHI mostly ranged from 0.55 to 0.70, and NHI from 0.55 to 0.70. The previous tight relationships among partitioning indices is also reflected when the plant S and P contents (entire season) are evaluated (with plant N content as bubble sizes, Fig. 5B). Maximum plant N, P, S, and K contents were all primarily governed by the N rate applied. Similar responses were reported previously (Kamprath, 1987; Wang et al., 2007; Setiyono et al., 2010; Mallarino et al., 2011).
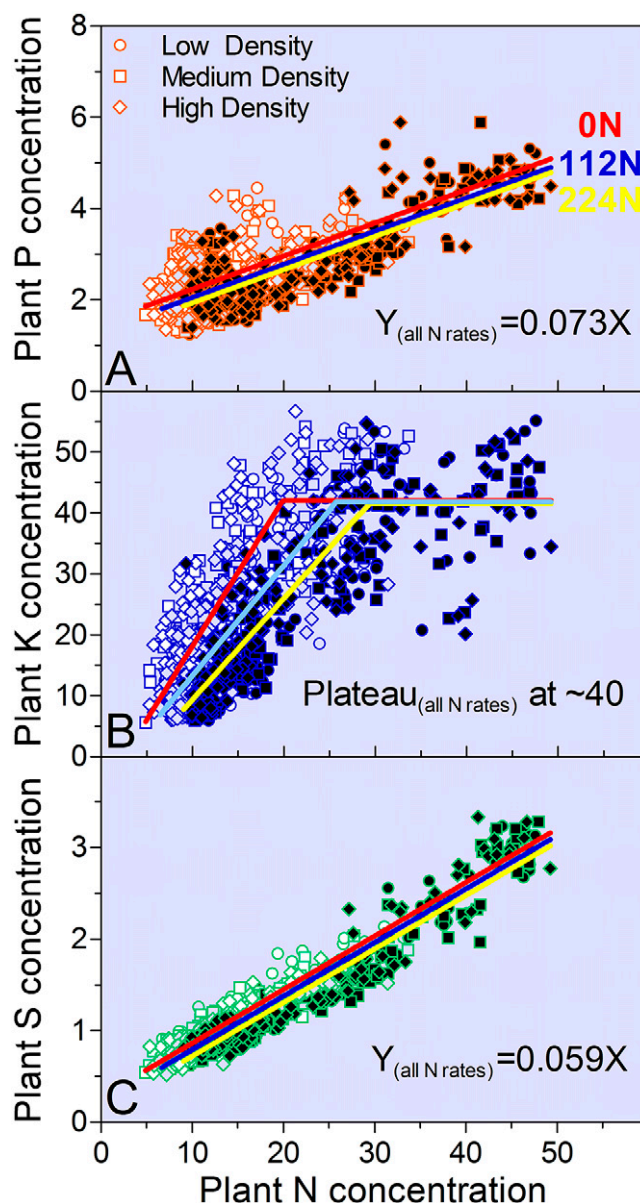
Nutrient Ratios: Nitrogen/Phosphorus, Nitrogen/Potassium, and Nitrogen/Sulfur

Whole-plant N/P, N/K and N/S content ratios were calculated for the entire season (Table 4). The overall N/P ratio began at ∼9 (V5) and progressively declined to ∼4 as the crop aged (R6). In addition, the N/P ratio was mostly affected by the N rate (plant P and N concentrations were linearly related, Fig. 6A). A very similar association between plant P and N concentrations for different herbage species, evergreen trees, and deciduous shrubs were observed by Duru and Ducrocq (1996), Wright et al. (2004), and Kerkhoff et al. (2006). This tight association between leaf N and P concentrations in different crops suggests that N and P follow a proportional association not only for maize but also for other

Table 4. Plant nutrient ratios for different maize growth stages (V5, V10, V15, R1, R3, and R6) at three plant densities and three N rates (0, 112, and 224 kg ha⁻¹) across two hybrids, locations, and growing seasons. Only statistical significant levels ($P < 0.05$) for single or interacting main factors (plant density [PD] and N rate [Nr]) resulting from the ANOVA analysis are presented. The standard error (SE) relates only to comparisons between significant terms. Nutrient ratios include all aboveground fractions (in vegetative stages, sum of leaves plus stem fractions; in reproductive stages, sum of shoot plus ear components [grain, husk, and cob]).

| | Vegetative stages | | | | | | | | | | Reproductive stages | | | | | | | | | |
| Plant N/P ratio/N/K ratio/N/S ratio | V5 stage | V10 stage | | | V15 stage | | | R1 stage | | | R3 stage | | | R6 stage | | |
Plant density†	Soil N	0N	112N	224N	0N	112N	224N	0N	112N	224N	0N	112N	224N	0N	112N	224N
PD1	9.2/1.00/15.6	6.3/0.53/13.1	8.4/0.72/16.2	9.3/0.78/17.4	5.7/0.47/12.9	7.2/0.62/14.8	8.3/0.71/16.7	5.6/0.57/12.7	6.9/0.73/14.4	7.9/0.85/16.0	4.4/0.76/10.6	5.2/0.94/11.5	5.8/1.02/12.8	3.8/0.98/10.7	4.5/1.21/11.6	4.7/1.38/12.4
PD2	9.3/1.02/15.7	6.1/0.49/13.3	8.2/0.65/16.3	9.5/0.81/17.8	5.6/0.46/13.0	7.1/0.56/14.9	8.4/0.75/17.0	5.3/0.53/12.4	6.6/0.65/14.2	8.0/0.84/16.0	4.5/0.68/10.9	5.3/0.82/11.9	6.1/1.02/13.2	3.6/0.91/10.5	4.0/1.07/11.4	4.7/1.34/12.5
PD3	9.6/1.03/14.7	6.2/0.48/13.0	8.5/0.69/15.8	9.5/0.75/17.4	5.6/0.45/13.2	7.2/0.54/14.3	8.5/0.67/16.4	5.5/0.51/12.5	7.0/0.65/14.1	8.4/0.80/16.4	4.6/0.66/10.6	5.5/0.81/12.4	6.2/0.95/13.4	3.9/0.91/10.9	4.5/1.02/11.3	5.2/1.26/12.7
ANOVA	PD/ns‡/PD	Nr/PD × Nr/Nr			Nr/PD, Nr/Nr			PD, Nr/PD, Nr/Nr			Nr/PD, Nr/Nr			PD, Nr/PD, Nr/Nr		
SE	0.11/–/0.13	0.15/0.019/0.13			0.15/0.012, 0.011/0.20			0.10/0.012, 0.011/0.21			0.11/0.018, 0.017/0.20			0.12, 0.11/0.018, 0.017/0.15		

† PD1, low = 54,000 plants ha⁻¹; PD2, medium = 79,000 plants ha⁻¹; PD3, high = 104,000 plants ha⁻¹.

‡ ns = not significant at $P = 0.05$.

Fig. 6. Association between plant (A) P, (B) K, and (C) S concentrations (g kg⁻¹ dry mass) vs. plant N concentration (g kg⁻¹ dry mass) from planting to maturity evaluated at three plant densities (low, 54,000 [circles]; medium, 79,000 [squares]; and high, 104,000 [diamonds] plants ha⁻¹) and three N rates (0, 112, and 224 kg N ha⁻¹ sidedressed) across two hybrids, sites, and growing seasons. Each observation represents a replication ($n = 1296$). Equations for (A) have the same slope for all N rates ($Y = 0.073X$), intercepts were 1.5 (0N), 1.3 (112N), and 1.2 (224N); for (B), the same plateau for all N rates (~40 g kg⁻¹), slopes were $2.4X$ (0N), $1.8X$ (112N), and $1.7X$ (224N); for (C), the same slope for all N rates ($Y = 0.059X$), intercepts were $0.3X$ (0N), $0.2X$ (112N), and $0.1X$ (224N).

species. Additionally, in agreement with Ziadi et al. (2007), a lack of N application affected the plant N concentration much more than the P concentration. Similar N/P dilution for maize and C_3 species was reported by Greenwood et al. (2008).

Furthermore, the N/P ratio can be understood from study of the dilution curves (Fig. 2). Thus, changes in the N/P are primarily related to the N dilution curve (a sharp decline in plant N for 0 > 112 > 224 kg N ha⁻¹; exponential coefficients [b] were –0.38, –0.28, and –0.24, respectively) rather than for P evolution (one model for all N rates; $b = -0.18$). Two points are noteworthy: (i)

at all N rates, N was proportionally more diluted than P as the crop aged; and (ii) as also stated by Greenwood et al. (2008), due to the differences in the declination rates (different *b* values), the N/P ratio should not be expected to be constant. At the plant level, the N/P ratio for cereals, legumes, and oilseeds crops was largely influenced by the P rather than the N content (Sadras, 2006). Ziadi et al. (2007) showed that the optimum N/P ratio is related to the N rate applied and the BM level. Similar N effects over P contents were documented by Kamprath (1987).

The N/K ratio was close to 1.0 at the early vegetative stage (V5) but declined rapidly to ~0.58 at V15 (Table 4). The N/K ratio also declined as PD increased and as the N rate decreased. A linear-plateau association was fitted for the plant K and N relationship between V5 and R6, and a unified plateau was evident close to ~40 g K kg^{-1} (Fig. 6B). At the plant level, modifications in the N/K ratio were more related to evolution in plant N than K (Fig. 2). The interaction between N and K is complex (Zhang et al., 2010). Under severe N deficiency (BM reduction >50%), different K dilution patterns were evident for 0 vs. 67 to 134 kg N ha^{-1} (Jordan et al., 1950), which was further exacerbated under higher PD (30,000 vs. 10,000 plants ha^{-1}). In addition, application of only N and P (under low soil K supply) increased BM and N content while depressing the plant K status (Wang et al., 2007).

The vegetative-stage N/S ratio remained stable (13–17) and increased as the N rate increased (Table 4). After flowering, the N/S ratio started to decline, reaching an overall value of ~11 at maturity (uniquely affected by N rate) (Table 4). Plant N and S concentrations showed a very tight association during the entire season, with a 1.14 faster dilution rate for N than for S (Fig. 6C). Similar results for the N and S dilution with time were documented by Chandler (1960). In fact, similar N/S ratios were reported not only for maize (Stewart and Porter, 1969; summarized by Sumner, 1978) but also for cereal and legume species (reviewed by Dijkshoorn and van Wijk, 1967). Several researchers have documented a critical leaf N/S ratio close to 15:1 (severe S limitation with leaf N/S ratio >20:1), which varied with the BM level (Dijkshoorn and van Wijk, 1967; Reneau, 1983) and the N rate applied (Fig. 6C). Lastly, four points are noteworthy: (i) the dilution model for plant N and S concentrations was unique for each N rate; (ii) declination rates (allometric exponents; Fig. 2C and 2D) were greater for N than S, except at the 224 kg N ha^{-1} level; (iii) isometric N/S ratios are feasible only under an optimum N supply; and (iv) under 0 N, the N/S ratio followed a dilution model with no buffer effect, while a buffer effect was observed when sidedress N (112–224 kg N ha^{-1}) was applied (Table 4).

CONCLUSIONS

The most striking findings from this research are: (i) P, K, and S contents at maturity were influenced differently by N rate; (ii) plant component partitioning with time revealed greater "buffering" propensity (i.e., smaller variations) for P, K, and S nutrient concentrations at the leaf vs. the stem (vegetative growth) and in the ear vs. the shoot (reproductive growth); (iii) the log–log analyses confirmed a unique stoichiometric ratio between components (for P and K contents in leaf vs. stem) and within the same component (N and S concentrations in the leaf, stem [vegetative], and ear [reproductive]); (iv) relative nutrient partitioning from plant to ear during the period bracketing

silking was P > S > N > K; (v) no minimum nutrient content per plant appeared to be required for the onset of P, K, or S allocation to ears; (vi) maximum nutrient partitioning rates near silking were comparable to final nutrient harvest indices at maturity, suggesting that maximum N, P, K, and S partitioning is genetically modulated; and (vii) nutrient ratio evolution during the entire season was primarily affected by the N rate, and all N/P, N/K, and N/S ratios increased with higher N applications.

The major conclusions regarding nutrient internal efficiencies were: (i) all internal efficiencies improved as the N rate increased but decreased as PD increased; and (ii) relative nutrient internal efficiencies for P, K, and S in response to N rates depended on the grain nutrient concentrations and harvest indices whereby (a) exponentially greater PIE occurred as the grain P concentration was reduced (similar to the NIE behavior), and (b) KIE was primarily explained by KHI (and stover K concentration) and SIE was positively and jointly governed by both grain S concentration and SHI.

Maize genotype or management advances that achieve superior BM and whole-plant N uptake seems to concomitantly result—at least in nonlimiting P, K, and S environments—in higher P, S, and K contents. Future research should study these nutrient balances under different stress conditions (e.g., N-limited environments, drought, or P, K, S, and micronutrient deficiencies). Further investigations of these macronutrient uptake and partitioning patterns in early breeding materials (populations or inbred lines) and a wider range of commercial hybrids will further improve our understanding of these complex macronutrient association traits under the umbrella of different genetic × environment × management combinations.

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